REMARKS

Upon entry of the amendment, claims 1-11, 13-14, 17-23, 41, 42 and 43 will be pending. A marked-up copy showing the amendment to the specification and the claims is attached as Exhibit A.

Claims 1, 3, 4, 6, 7, 9, 10, 11, 13, 14, 17, and 19-23 have been amended and new claims 41-43 has been added. No new matter has been added with the amendments or new claims. Newly added claim 41 is based on the portion of claim 12 that covers the elected invention. Newly added claim 42 is supported by the disclosure as filed, for example at page 43, last full sentence. Newly added claim 43 is supported by the disclosure as filed, for example claim 20. The amendment to the FIG.1 explanation is supported by the disclosure as filed, for example, FIG. 1 which indicates that the sequence disclosed therein is SEQ ID NO:1, and the specification at page 11, paragraph starting at line 14 which discloses the nucleotide positions of the enhancer elements as indicated.

It is acknowledged that the Examiner deemed claims 8 and 11 free of the prior art.

Specification

The Office Action objects to the specification in alleging that FIG. 1 is unclear in delineating the location of the enhancer within the first intron. The specification at page 29 has been amended to modify the figure explanation of figure 1 to include more details regarding the enhancer found within the first intron. Therefore, the Office Action's objection to the specification has been overcome.

Claim Rejection under 35 U.S.C. 112, Second Paragraph

Claims 1-11, 13, 14, 17-24, 34 and 35 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Office Action rejected claim 1 and dependent claims 2-11, 13, 14, 17-24, 34 and 35, alleging that the metes and bounds of "...an intron of a gene homologous to..." is unclear. Claim 1 as amended, does not include the

phrase objected to in the Office Action. Therefore, the rejection of claim 1 and dependent claims 2-11, 13, 14, 17-24, 34, and 35 is mute.

Claims 1-11, 13, 14, 17-24, 34 and 35 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention in reciting the term "capable." Claim 1 as amended no longer includes the term "capable," but indicates that the regulatory sequence confers expression in endothelial cells *in vivo*. Therefore, the rejection of claim 1 and dependent claims 2-11, 13, 14, 17-24, 34, and 35 has been overcome.

Claims 3 and 9 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention in reciting ". . . sequence which is conserved in. . ." and "anologue or derivative of a nucleotide sequence." These phrases have been deleted from claims 3 and 9. Therefore, the rejection of claims 3 and 9 have been overcome.

Claim 13 and 14 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention in being dependant from non-elected claim 12. Claim 13 and 14 have been amended to depend from newly added claim 41 which is directed to elected subject matter. Therefore, the rejection of claim 13 and 14 has been overcome.

Claim Rejection under 35 U.S.C. 112, First Paragraph

Claims 1-11, 13, 14, 17-24, 34 and 35 were rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention, citing *Vas-Cath Inc. v. Mahurkar*, 19USPQ2d 1111 (Fed. Cir. 1991). The Office Action alleges that the Applicants have not conveyed with reasonable clarity to those skilled in the art that, as of the filing date sought, they were in possession of the invention. The Office Action acknowledges that the specification provides adequate written description for the claimed invention (methods and

products) with regard to the regulatory region within the first intron of the murine VEGF flk-1 receptor. However, the Office Action alleges that the specification fails to describe the other species within the genus of regulatory sequences encompassed in the claims with particularity to indicate that applicants had possession of the claimed invention.

The Office Action asserts that the claimed embodiments of any and all regulatory sequences other than those specifically described within the first intron of the murine VEGF flk-1 receptor, lack a written description. The Office Action alleges that the specification fails to describe what elements other than those isolated from mouse, fall into the claimed genus. Furthermore, the Office Action alleges that it was unknown as of Applicants' effective filing date that any of these regulatory sequences would have the properties of murine VEGF flk-1 receptor. The skilled artisan, according to the Office Action, cannot envision the detailed chemical structure of all of the encompassed transcriptional regulatory sequences isolated from any and all species, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method citing *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016 (Fed. Cir. 1991).

The pending claimed inventions are adequately described by the disclosure as filed. The invention of pending claim 1, from which all other claims depend, is directed to recombinant DNA molecules that include a first regulatory sequence that confers *in vivo* expression in endothelial cells of an operatively linked heterologous DNA sequence. The first regulatory sequence has a structure that includes the nucleotide sequence of SEQ ID NO:1 which includes the mouse FLK-1 gene promoter and enhancer (Pg. 34, first full paragraph), the nucleotide sequence of the recited regions within SEQ ID NO:1 which are identified as enhancer regions in the present specification, nucleotide sequences which hybridize to the SEQ ID NO:1 and/or the specific enhancer sequences, and/or fragments of any of these sequences that confer expression in endothelial cells *in vivo*. Therefore, the detailed chemical structure of all of the encompassed regulatory sequences are known. That is, one of ordinary skill in the art can determine whether a nucleotide sequence falls within the structural limitations of the claim

for the regulatory sequence, and can then test the nucleotide sequence using known techniques, including those included in the Examples of the present specification, to determine whether it directs *in vivo* endothelial cell expression of a heterolgous DNA sequence.

As indicated above, the Office Action alleges that the specification fails to describe what elements other than those isolated from mouse, fall into the claimed genus. The pending claims, as well as the specification as filed, describe regulatory sequences other than those isolated from mouse that fall into the claimed genus. These include sequences that hybridize under stringent conditions and fragments of these sequences. One of ordinary skill in the art can determine the structure of any sequence that meets the claimed structural limitations using known hybridization methods or using mathematical equations that predict hybridization of nucleotide sequences under specified conditions. One of ordinary skill can use known methods, including those described in the present specification, to test whether regulatory sequences that meet these structural requirements, confer *in vivo* expression in endothelial cells on a heterologous DNA sequence. That is, the skilled artisan can envision and determine the detailed chemical structure of all of the possible first regulatory sequences.

Unlike the claims at issue in *Fiers v. Revel and Amgen v. Chugai*, the pending claims include a structural limitation. The claims held that the courts in *Fiers* and *Amgen* held were not enabled according to 35 U.S.C. 112, first paragraph were directed to DNA sequences encoding human fibroblast interferon-beta polypeptide and human erythropoieten (EPO), respectively, without encoding a structural limitation. These courts held that a structural limitation was required for the claimed inventions at issue to meet 35 U.S.C. 112, first paragraph requirements. With respect to the present application, the pending claims include a structural limitation. Furthermore, the Fiers' patent application provided no sequence information regarding human fibroblast interferon-beta polypeptide (Fiers at 1604), and although Amgen's EPO patent application provided sequence information regarding a few analogs, even after 5 years of experimentation the patentee could not determine which of those analogs had EPO activity (Amgen at 1027). The disclosure of the pending application provides a number of regulatory sequences that fall within the claims, including tightly defined

enhancer regions. The skilled artisan recognizes that many sequences that hybridize under stringent conditions with SEQ ID NO:1, or with specific enhancer regions within SEQ ID NO:1 will retain enhancer activity.

It is noteworthy that despite the Applicants contention that claims that encompass regulatory sequences that hybridize with the recited elements under stringent conditions are adequately described by the disclosure as filed, claim 3 is directed to only mouse sequences that have nucleotide sequences that are provided in the specification, or fragments of these sequences. Therefore, with respect to this claim, the written description requirement of 35 U.S.C. 112, first paragraph, is even more clearly met.

The Office Action rejected claims 20-23, 24, 34 and 35 under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Claims 24, 34, and 35 were rejected because the Office Action alleges that they are directed to the unproven field of gene therapy. Claims 24, 34, and 35 have been cancelled by the present invention to expedite allowance of the pending claims without prejudice to refiling the claims in a continuation application. Claims 20-23 were rejected because they encompass cells both *in vitro and in vivo* cells. Applicants amend these claims herein to identify the cells as "isolated," as suggested by the Examiner. Therefore, the rejection to claims 20-23, 24, 34, and 35 under 35 U.S.C. 112, first paragraph, has been overcome.

Claim Rejection under 35 U.S.C. 102

Claims 1-7, 9, 10, 13, 14, 17, 18, 20, 21, 22 and 24 were rejected under 35 U.S.C. 102(b)as being anticipated by Patterson et al. (WO97/00957). To anticipate an invention, each and every element of a claim must be found in a single prior art reference. MPEP 2131; Verdegaal Bros. V. Union Oil Co. of California, 814 F.2d 628,631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). As a preliminary matter, the Office Action indicates that the breadth of claims 1-7, 9, 10, 13, 14, 17, 18, 20, 21, 22 and 24 of the instant application is unacceptable because of

inclusion of the clause directed to a regulatory sequence of a gene homologous to the Flk-1 gene. The Office Action asserted that the originally filed claimed invention included regulatory sequences of tyrosine kinase type receptors which bind vascular endothelial growth factor (VEGF) such as flt-1. In order to expedite allowance of the pending application and without prejudice regarding the contents of claims of continuations, the clause directed to a gene homologous to Flk-1 has been deleted from claim 1. Therefore, the Office Actions rejections of the claims based on Flt-1 prior art has been overcome.

In fact, the pending claims are not anticipated by any prior art reference including Patterson et al. As indicated above, claims 1 and the remainder of claims, which are dependent on claim 1, require that the regulatory sequence that confers expression in endothelial cells *in vivo*. Patterson et al. does not disclose regulatory sequences which confer expression in endothelial cells *in vivo*. Patterson et al. identifies the promoter of the human flk-1 gene using experiments performed *in vitro* (page 11, line 31-page 12, line 28). Patterson et al. does not show that the promoters identified therein confer endothelial cell expression *in vivo*. Furthermore, Patterson et al. indicates that the human Flk-1 promoter shares important transcription factor binding sites with the mouse Flk-1 promoter (Patterson et al., page 18, lines 8-26), which is shown in the present specification not to be capable by itself of directing expression to endothelial cells *in vivo* (Present specification at page 39, first full paragraph). *In vivo* endothelial expression is conferred as a result of the presence of the enhancer elements identified in the present application (See e.g. present specification, Example 4). These enhancer elements are not present in Patterson et al.

The Office Action points out that Patterson et al. discloses a portion of the first intron of human Flk-1. Although FIG 1 of Patterson et al. possibly discloses the 19-most 5' nucleotides of the first intron of human Flk-1, such sequences are not the first regulatory sequence recited in the pending claims. In fact, the 5' portion of the first intron of the mouse Flk-1 gene ("5'-In1" in Fig. 4) which includes a much larger portion of the first intron than the Patterson et al. construct, did not contain the *in-vivo* enhancer element of the invention (Pg. 41, last sentence). Therefore, Patterson et al. does not disclose the first regulatory sequence, and therefore it does

not anticipate the present invention. It is further noted regarding newly added claim 42, that Patterson et al. is silent as to regulatory sequences that confer endothelium-specific expression *in vivo* of a heterologous DNA sequence

Claim Rejections – 35 USC § 103

Claims 19 and 23 were rejected under 35 U.S.C. 103(a) as being unpatentable over Patterson et al. and Ema et al. To establish a *prima facie* case of obviousness there must be some suggestion or motivation in the prior art to make the claimed invention, there must be a reasonable expectation of success, and the prior art reference must teach or suggest all of the claim limitations. MPEP 2142; In re Vaeck, 947 F.2d 488, 20 USPQ2d, 1438 (Fed. Cir. 1991). As discussed above, Patterson et al. does not disclose a first regulatory sequence. The first regulatory element is also not disclosed or suggested by Ema et al.. Rather, Ema et al. is directed to a hypoxia inducible factor, the bHLH-PAS factor. Since neither Patterson et al. nor Ema et al. either singly or in combination teach, suggest, or motivate a first regulatory sequence according to the pending claims, they do <u>not</u> render any of the pending claims obvious.

In view of the amendments and the above remarks, it is submitted that the claims are in condition for allowance and a notice to that effect is respectfully requested. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this application.

Please charge any additional fees, or make any credits, to Deposit Account No. 50-1355.

Respectfully submitted,

Date: January 17, 2002

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Enclosures: Exhibit A

EXHIBIT A

MARKED-UP COPY OF THE SPECIFICATION AND THE CLAIMS SHOWING THE AMENDMENTS

A. In the Specification

Please amend the third paragraph on page 29, the paragraph which describes Figure 1, as follows:

Nucleotide sequence of the murine Flk-1 gene (SEQ ID NO:1). The ATG codon is at position +299. The three exons are indicated in bold. Motifs for transcription factors are underlined. VRE: vascular response element. Enhancer elements of the present invention that confer expression in endothelial cells are found in the first intron (nucleotides 7027 to 10642). These enhancer elements include the nucleotide sequence from nucleotide 8260 to nucleotide 10560, from nucleotide 8336 to nucleotide 10608 and/or from nucleotide 10094 to nucleotide 10608.

B. In the Claims

Please cancel claims 24, 34, and 35.

Please amend claims 1, 3, 4, 6, 7, 9, 10, 11, 13, 14, 17, and 19-23 to read as follows:

1. (Amended) A recombinant DNA molecule comprising:

(a) at least one first regulatory sequence [of an intron of the Vascular Endothelial Growth factor (VEGF) receptor-2 (Flk-1) gene] which confers [or of a gene homologous to the Flk-1 gene being capable of conferring] expression in endothelial cells in vivo, wherein said first regulatory sequence is selected from the group consisting of

(i) DNA sequences comprising a nucleotide sequence as given in SEQ ID NO: 1;

(i) DNA sequences comprising a nucleotide sequence of SEQ ID NO: 1 from nucleotide 8260 to nucleotide 10560, from nucleotide 8336 to nucleotide 10608 and/or from nucleotide 10094 to nucleotide 10608;

(iii) DNA sequences comprising a nucleotide sequence which hybridizes with a nucleotide sequence of (i) or (ii) under stringent conditions; and

(iv) DNA sequences comprising a fragment of a nucleotide sequence of (i) (ii), or (iii); and

(b) operatively linked thereto a heterologous DNA sequence.

3. (Amended) The recombinant DNA molecule of claim 1 or 2, wherein said first regulatory sequence is selected from the group consisting of

(a) DNA sequences comprising a nucleotide sequence as given in SEQ ID NO: 1;

(b) DNA sequences comprising a nucleotide sequence of SEQ ID NO: 1 from nucleotide 8260 to nucleotide 10560, from nucleotide 8336 to nucleotide 10608 and/or from nucleotide 10094 to nucleotide 10608; and

(c) [DNA sequences comprising the nucleotide sequence of the human Flk-1-intron;

(d) DNA sequences comprising a nucleotide sequence which hybridizes with a nucleotide sequence of (a), (b) or (c) under stringent conditions;

(e) DNA sequences comprising a nucleotide sequence which is conserved in the nucleotide sequences of (a), (b) and (c); and]

(f)] DNA sequences comprising a fragment[, analogue or derivative] of a nucleotide sequence of any one of (a) to (c) [(e)] capable of conferring expression in endothelial cells.

4. (Amended) The recombinant DNA molecule of any one of claims 1 to [3] 2, wherein said heterologous DNA sequence is operatively linked to further regulatory sequences.

6. (Amended) The recombinant DNA molecule of claim 4 [or 5], wherein said further regulatory sequence is a 3'-untranslated region.

7. (Amended) The recombinant DNA molecule of claim 5 [or 6], wherein said promoter is a promoter of hypoxia inducible genes, genes encoding growth factors or its receptors or glycolytic enzymes.

9. (Amended) The recombinant DNA molecule of [any one of claims 5 to 8] claim 5, wherein said promoter comprises a DNA sequence selected from the group consisting of

(a) DNA sequences comprising the nucleotide sequence as given in SEQ ID NO: 1 from nucleotide 6036 to nucleotide 6959;

(b) DNA sequences comprising the nucleotide sequence of the human Flk-1/KDR promoter;

(c) DNA sequences comprising a nucleotide sequence which hybridizes with a nucleotide sequence of (a) or (b) under stringent conditions; and

(d) [DNA sequences comprising a nucleotide sequence which is conserved in the nucleotide sequences of (a) and (b); and

(e)] DNA sequences comprising a fragment[, analogue or derivative] of a nucleotide sequence of any one of (a) to (c) [(d)].

10. (Amended) The recombinant DNA molecule of any one of claims 1 to [9] 2, wherein at least one of said DNA sequences is of human or murine origin.

11. (Amended) The recombinant DNA molecule of any one of claims 1 to [10] 2, wherein said heterologous DNA sequence being operatively linked to said regulatory sequences is located 5' to said first regulatory sequence.

13. (Amended) The recombinant DNA molecule of claim 41 [12], wherein said protein is selected from the group consisting of Vascular Endothelial Growth Factor (VEGF), Hypoxia Inducible Factors 7(HIF), HIF-Related Factor (HRF), tissue plasminogen activator, p21 cell

cycle inhibitor, nitric oxide synthase, interferon-γ, atrial natriuretic polypeptide and monocyte chemotactic proteins.

14. (Amended) The recombinant DNA molecule of claim 41 [12], wherein said protein is a scorable marker, preferably luciferase, green fluorescent protein or lacZ.

17. (Amended) A vector comprising a recombinant DNA molecule of any one of claims 1 to [15] 2.

19. (Amended) The vector of claim 17 [or 18], further comprising a gene capable of expressing HIF-2α.

20. (Amended) An isolated [A] cell transformed with a DNA molecule of any one of claims 1 to [15] 2 [or the vector of any one of claims 17 to 19].

21. (Amended) The isolated cell of claim 20, which is a prokaryotic or eukaryotic cell.

22. (Amended) The isolated cell of claim 20 [or 21], which is an endothelial cell.

23. (Amended) The isolated cell of [any one of claims] claim 20 [to 22], further comprising a recombinant DNA molecule or vector containing a gene capable of expressing HIF-2α.

Please add the following claim:

--41. The recombinant DNA molecule of any one of claims 1 to 2, wherein said heterologous DNA sequence encodes a peptide, protein, sense RNA, or ribozyme.

42. The recombinant DNA molecule of claim 1, wherein the first regulatory sequence confers endothelium-specific expression *in vivo* of the heterologous DNA sequence.

43. An isolated cell transformed with the vector of claim 17.--